

CLAIMS

What is claimed is:

1. A substantially pure human mitogen-activated protein kinase kinase (MKK) polypeptide having serine, threonine, and tyrosine kinase activity, and phosphorylating human mitogen-activated protein (MAP) kinase p38.

2. A polypeptide of claim 1 comprising the amino acid sequence of SEQ ID NO:2.

3. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 2.

4. An isolated and purified polynucleotide sequence of claim 3 consisting of the sequence of SEQ ID NO:1 or degenerate variants thereof, or a polynucleotide sequence fully complementary to the sequence of SEQ ID NO:1 or degenerate variants thereof.

5. An isolated and purified polynucleotide sequence of claim 3 consisting of a polynucleotide sequence that hybridizes under stringent hybridization conditions to the sequence of SEQ ID NO:1.

6. A recombinant expression vector containing a polynucleotide sequence of claim 3.

7. A recombinant host cell comprising a polynucleotide sequence of claim 3.

8. A polypeptide of claim 1 comprising the amino acid sequence of SEQ ID NO:4.

9. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 8.

10. An isolated and purified polynucleotide sequence of claim 3 consisting of the sequence of SEQ ID NO:3 or degenerate variants thereof, or a polynucleotide sequence fully complementary to the sequence of SEQ ID NO:3 or degenerate variants thereof.

11. An isolated and purified polynucleotide sequence of claim 9 consisting of a polynucleotide sequence that hybridizes under stringent hybridization conditions to the sequence of SEQ ID NO:3.

12. A recombinant expression vector containing a polynucleotide sequence of claim 9.

13. A recombinant host cell comprising a polynucleotide sequence of claim 9.

14. A polypeptide of claim 1, further characterized in that said polypeptide phosphorylates human mitogen-activated protein (MAP) kinase JNK.

15. A polypeptide of claim 14 comprising the amino acid sequence of SEQ ID NO:6.

16. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 15.

17. An isolated and purified polynucleotide sequence of claim 16 consisting of the sequence of SEQ ID NO:5 or degenerate variants thereof, or a polynucleotide

sequence fully complementary to the sequence of SEQ ID NO:5 or degenerate variants thereof.

18. An isolated and purified polynucleotide sequence of claim 16 consisting of a polynucleotide sequence that hybridizes under stringent hybridization conditions to the sequence of SEQ ID NO:5.

19. A recombinant expression vector containing a polynucleotide sequence of claim 16.

20. A recombinant host cell comprising a polynucleotide sequence of claim 16.

21. A polypeptide of claim 14 comprising an amino acid sequence of SEQ ID NO:8.

22. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 21.

23. An isolated and purified polynucleotide sequence of claim 22 consisting of the sequence of SEQ ID NO:7 or degenerate variants thereof, or a polynucleotide sequence fully complementary to the sequence of SEQ ID NO:7 or degenerate variants thereof.

24. An isolated and purified polynucleotide sequence of claim 22 consisting of a polynucleotide sequence that hybridizes under stringent hybridization conditions to the sequence of SEQ ID NO:7.

25. A recombinant expression vector containing a polynucleotide sequence of claim 22.

26. A recombinant host cell containing a polynucleotide sequence of claim 22.

27. A polypeptide of claim 14 comprising the amino acid sequence of SEQ ID NO:10.

28. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 27.

29. An isolated and purified polynucleotide sequence of claim 28 consisting of the sequence of SEQ ID NO:9 or degenerate variants thereof, or a polynucleotide sequence fully complementary to the sequence of SEQ ID NO:9 or degenerate variants thereof.

30. A recombinant expression vector comprising a polynucleotide sequence of claim 28.

31. A recombinant host cell comprising a polynucleotide sequence of claim 28.

32. A purified antibody which binds specifically to a polypeptide of claim 1.

33. A purified antibody which binds specifically to a polypeptide of claim 2.

34. A purified antibody which binds specifically to a polypeptide of claim 8.

35. A purified antibody which binds specifically to a polypeptide of claim 14.

36. A purified antibody which binds specifically to a polypeptide of claim 15.

37. A purified antibody which binds specifically to a polypeptide of claim 21.

38. A purified antibody which binds specifically to a polypeptide of claim 27.

39. A method of measuring the activity of a mitogen-activated protein kinase kinase (MKK) in a biological test sample, said method comprising:

a) incubating said test sample with an MKK substrate for the MKK polypeptide of claim 1 and labeled phosphate under conditions sufficient to allow phosphorylation of said substrate, and

b) determining the rate of incorporation of labeled phosphate into said substrate, wherein said rate of incorporation is a measure of MKK activity.

40. A method of claim 39 wherein said MKK substrate is selected from the group consisting of p38 and JNK MAP kinases, activating transcription factor-2 (ATF2), ATFa, cAMP response element binding protein (CRE-BPa), and c-Jun.

41. A method of claim 39 wherein said biological test sample is fluid, cells, or tissue obtained from a mammal.

42. A method for measuring the synthesis of MKK in a biological test sample, comprising the steps of:

a) fractionating proteins present in said sample by gel electrophoresis;

b) transferring the proteins onto a membrane; and
c) probing the proteins with a labeled antibody specific to a MKK polypeptide of claim 1, wherein the level of MKK synthesis is determined by the amount of bound labeled antibody.

43. A method for measuring the level of expression of MKK in a test sample, comprising the steps of:

- a) isolating polyadenylated RNA from the test sample;
- b) incubating polyadenylated RNA with a polynucleotide probe specific for a MKK polypeptide of claim 1;
- c) determining the amount of said probe hybridized said polyadenylated RNA, wherein the level of expression of MKK is directly related to the amount of MKK probe hybridized to said RNA.

44. A method for identifying a reagent which modulates MKK activity, said method comprising:

- a) using the method of claim 39;
- b) comparing the effect of said reagent on MKK activity relative to a control, wherein a reagent able to modulate MKK substrate phosphorylation is identified.

45. A method of claim 44 wherein said MKK substrate is one or more of p38, JNK, ATF2, ATFα, CRE-BPa, and c-Jun.

46. A method of claim 44 wherein said modulation is inhibition of MKK activity.

47. A method for identifying a reagent which modulates MKK synthesis, said method comprising:

- a) using the method of claim 42;
- b) comparing the effect of said reagent on MKK synthesis relative to a control, wherein a reagent able to modulate MKK synthesis is identified.

48. A method of claim 47 wherein said MKK substrate is one or more of p38, JNK, ATF2, ATFa, CRE-BPa, and c-Jun.

49. A method of claim 47 wherein said modulation is inhibition of MKK synthesis.

50. A method for identifying a reagent which modulates MKK expression, said method comprising:

- a) using the method of claim 43;
- b) comparing the effect of said reagent on MKK expression relative to a control, wherein a reagent able to modulate MKK expression is identified.

51. A method of treating an MKK-mediated disorder in a patient, comprising administering to the patient a therapeutically effective amount of a reagent that modulates MKK activity.

52. The method of claim 51, wherein said MKK-mediated disorder is selected from the group consisting of ischemic heart disease, kidney failure, oxidative liver damage, respiratory distress syndrome, heat and radiation burns, septic shock, rheumatoid arthritis, autoimmune disorders, and inflammatory diseases.

53. A kit useful for the detection of MKK, said kit comprising a buffer and a reagent which binds to a MKK polypeptide of claim 1, wherein said a sample to be tested

is mixed with said buffer and said reagent, and wherein said reagent is labeled.

54. A kit of claim 53, wherein said reagent is an antibody which specifically binds MKK.

55. A kit of claim 53, wherein said reagent is a labeled cDNA oligonucleotide which specifically bind MKK mRNA.